Invited Review

Role of myofibroblasts during normal tissue repair and excessive scarring: Interest of their assessment in nephropathies

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Summary. Following injury, tissue repair process takes place involving inflammation, granulation tissue formation and scar constitution. Granulation tissue develops from the connective tissue surrounding the damaged area and contains vessels, inflammatory cells, fibroblasts and myofibroblasts. Myofibroblasts play an important role in many tissue injuries and fibro-contractive diseases. The process of normal wound repair after tissue injury follows a closely regulated sequence including the activation and the proliferation of fibroblastic cells. In pathological situations, the normal resolution stages are abrogated and the proliferation of myofibroblasts continues, inducing excessive accumulation of extracellular matrix. The differentiation of fibroblastic cells into myofibroblasts is an early event in the development of tissue fibrosis. Myofibroblastic cells express smooth muscle cytoskeletal markers (α-smooth muscle actin in particular) and participate actively in the production of extracellular matrix. The evaluation of myofibroblast differentiation in renal biopsies would be useful for histopathologists to appreciate the intensity of tissue injury and particularly to predict the long term outcome of some nephropathies. Immunohistochemical studies for a-smooth muscle actin should be made systematically in renal tissue biopsies. Myofibroblastic differentiation appears to play a significant role in the progression of renal failure and seems to be a useful marker of progressive disease.

Key words: Myofibroblast, Fibrosis, Kidney, Cytoskeleton, a-Smooth muscle actin

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introduction

During tissue repair and fibrocontractive diseases, fibroblastic cells participating in granulation tissue development acquire some morphological and biochemical features of smooth muscle (SM) cells (Schürch et al., 1992). These modified fibroblastic cells, called myofibroblasts, express SM cytoskeletal markers (a-SM actin in particular) and are involved in tissue contraction. Furthermore, they participate actively in the production of extracellular matrix (Desmoulière and Gabbiani, 1996). Since 1971, when myofibroblastic cells were first described by Gabbiani et al., it has been shown that these cells play a major role during normal tissue repair but also in pathological processes such as fibrosis in various organs (e.g. lungs, liver, and kidneys). Fibrosis is a common consequence of abnormal healing process in response to various exogenous insults (physical, chemical or biological). The mechanisms of induction and progression of fibrosis are complex and involve multiple cellular interactions via cytokines and growth factors.

Renal fibrosis is the final common pathway for almost all forms of kidney disease that progress to end stage of renal failure. Progressive renal disease involves both glomerular and interstitial processes. In glomeruli, sclerosis occurs with progressive deposition of extracellular matrix components that reduce the filtration surface area. Most research on progressive glomerular disease has focused on changes in glomerulus. However, tubulointerstitial changes correlate closely with the loss of renal function in patients with glomerular diseases (Bohle et al., 1996). In several glomerular diseases such as membranous nephropathy, endocapillary acute glomerulonephritis or diabetic nephropathy, the long term renal outcome correlates strongly with the development of interstitial fibrosis (Austin et al., 1994; Bohle et al., 1996; Ziyadeh, 1996). During experimental

and human glomerulonephritis, mesangial cells and interstitial fibroblasts acquire α-SM actin expression (Alpers et al., 1992; Tang et al., 1997). The proliferation of mesangial cells and resident interstitial fibroblasts and their differentiation into myofibroblasts are early events in the development of kidney fibrosis (Desmoulière and Gabbiani, 1995). The prognostic significance of α-SM actin expression seems to be important in the evaluation of the progression of pathological situations including renal failure and could have a clinical utility in the therapeutic management. After general considerations on cytoskeletal features of fibroblastic cells, we will present tissue repair mechanisms and the cells involved in kidney fibrosis; we will also discuss the prospective interest of myofibroblastic evaluation during the progression of kidney diseases.

Cytoskeletal features of fibroblastic cells

Cytoskeletal proteins represent useful markers of cell differentiation and the study of their expression allows the characterization of fibroblastic phenotypic modifications corresponding to functional changes which take place during physiological and pathological repair processes (Sappino et al., 1990).

The cytoskeleton of eukaryotic cells is built on a framework of three types of filaments. The intermediate filaments, which are strong and durable, strengthen cells against mechanical stress; their composition depends on cell types. In mesenchymal cells, they are homopolymer of vimentin or desmin in the cytoplasm and of lamins in the inner surface of the nuclear membrane (Raats and Bloemendal, 1992). The microtubules are hollow tubes which play a crucial role in cell organization and drive intracellular transport via motor proteins. They consist mainly of tubulin. Finally, the microfilaments are thin and flexible, essential for many movements and made up essentially of actin.

Actin is not a unique protein but exists as six different isoforms: two cytoplasmic or non-muscle actins (ß and γ), and four muscle actins (two striated muscle actins, α-skeletal and α-cardiac, and two SM actins, α and γ) (Vandekerckhove and Weber, 1978; Buckingham et al., 1984). The six isoactins are encoded by a separate gene, each having a distinct stage- and tissue-specific pattern of expression during development. The protein sequence of actin isoforms shows a very high degree of homology (93.5 to 99.5%) and is strictly conserved in humans, rodents and chickens (Sheterline and Sparrow, 1994).

In spite of their strong similarity, isoactins seem not to be interchangeable in vivo but rather reflect a functional diversity. Muscle actins resemble each other more than non-muscle actins and recent studies have argued for differences in their respective structural and biochemical properties (Allen et al., 1996; Rønnov-Jessen and Petersen, 1996; Mounier and Sparrow, 1997; Qu et al., 1997). Several isoactins co-exist within a single cell but are differentially located (Rubenstein,

1990; Herman, 1993; North et al., 1994). When they are transfected into different cell types, muscle and non-muscle actins are differentially sorted and utilized by the cellular machinery (Schevzov et al., 1992; vonArx et al., 1995; Mounier et al., 1997, 1999), supporting the assumption of their functional diversity.

Actin filament is a two-stranded helix of actin monomer and can associate with a myriad of actin-binding proteins (capping, severing, crosslinking, bundling, nucleating, sequestering or motor proteins) that modify and enable the filament to perform a variety of functions. Myosins are actin-based motor protein and hydrolyse ATP providing the energy for their movement along actin filaments. A large variety of isoforms is present in muscle (striated or smooth) and non-muscle cells. Conventional or class II myosins are hexamers of two heavy chains (MHC) and two pairs of light chains (MLC) (Schiaffino and Reggiani, 1996; Loukianov et al., 1997).

The analysis of cytoskeletal elements has been facilitated by the production of specific antibodies for vimentin and desmin (Franke et al., 1978; Gard et al., 1979; Osborn et al., 1981; Kocher et al., 1984), SM and non-muscle MHC isoforms (Benzonana et al., 1988; Eddy et al., 1988; Giuriato et al., 1992), and actin isoforms (Gown et al., 1985; Skalli et al., 1986, 1989; Tsukada et al., 1987), particularly a monoclonal antibody against the α-SM actin (Skalli et al., 1986).

Another useful approach is the analysis of gene regulation by detection of messenger RNAs (mRNA) using specific molecular probes. For instance, α-SM actin gene expression has been studied at the level of RNA accumulation by an RNA-specific probe in SM cells and fibroblasts (Barret and Benditt, 1987; Kocher and Gabbiani, 1987; Bochaton-Piallat et al., 1992). Transcriptional regulation of the α-SM actin and SM-MHC genes is complex and depends on several positive and negative cis-acting elements located in the promotor region. Other functional elements have been identified in the first intron of both genes. The α-SM actin gene is furthermore differentially regulated in SM *versus* non-SM cells. Isoforms of SM-MHC, but not actin isoforms, derived from alternative splicing of a single gene (Owens et al., 1996; White and Low, 1996; Madsen et al., 1998).

Numerous recent studies have illuminated our understanding of dynamics of actin cytoskeleton. Assembly and disassembly of actin filament bundles result from the association of various actin-binding proteins and are regulated by small GTPases related to the Ras superfamily. Rearrangements of actin cytoskeleton, particularly at the surface membrane, mediate different cellular processes. Rho GTPases for instance, in response to extracellular signals, regulate the formation of stress fibers, the contractile actin-myosin filaments, and focal adhesion complexes (Machesky and Hall, 1996; Molitoris, 1997; Hall, 1998). Markers of actin cytoskeleton, particularly α-SM actin in fibroblastic and myofibroblastic cell populations, are

thus good tools to evaluate phenotypic modifications in response to physiological or pathological situations.

General comments on tissue repair

Mechanisms of tissue repair

After injury, a series of events takes place to repair the damaged tissue. These events are well characterized, and may be divided into three main steps: inflammatory phase; granulation tissue development and scar formation. The inflammatory phase begins at the moment of injury. As a consequence of trauma, a disruption of blood vessels occurs and there is clot formation. The platelets participating in clot formation release many substances such as cytokines and growth factors. These substances will act as chemotatic and/or mitogenic agents for leukocytes and other cells (e.g. endothelial cells, fibroblasts). The first leukocytes that invade the lesion are neutrophils followed by mono-cytes/macrophages, which also produce many kinds of cytokines and growth factors implicated particularly in fibroblast proliferation and migration. Soon afterwards the granulation tissue formation starts and is characterized by fibroblast proliferation, angiogenesis and extracellular matrix deposition. Granulation tissue fibroblasts acquire some SM features that characterize the myofibroblast (for review, see Desmoulière and Gabbiani, 1996; Schürch et al., 1998). Finally, scar formation implicates tissue remodeling with extra-cellular matrix degradation and decrease of the cellularity. In some cases (i.e. non healing wounds or excessive scarring) problems occur and lead to the development of pathological situations (Schmitt-Gräff et al., 1994).

Myofibroblasts

The activated fibroblasts, called myofibroblasts, are the main cellular type present in granulation tissue (Gabbiani, 1998). They contain cytoplasmic bundles of microfilaments or stress fibers which, as in SM cells, play a role in contraction (Grinnell, 1994). Myofibro-blasts are interconnected by gap junctions and are also connected to the extracellular matrix, for example by the fibronexus, a transmembrane complex involving intra-cellular microfilaments in continuity with extracellular fibronectin fibers (Singer et al., 1984).

Myofibroblasts express different sets of cytoskeletal proteins. The presence of α-SM actin has been the first marker to identify and characterize these cells (Darby et al., 1990) and is related to its contractile phenotype. Well differentiated myofibroblasts express SM-MHC in agreement with their capability to produce the contraction force (Buoro et al., 1993; Chiavegato et al., 1995). Myofibroblasts also express vimentin and/or desmin. Using these cytoskeletal markers, they have been classified into four types of myofibroblastic populations expressing, in addition to the two cytoplasmic actins, 1) vimentin or V-type, 2) vimentin and desmin or VD-type, 3) vimentin and α-SM actin or VA-type, and 4) vimentin, desmin and α-SM actin or VAD-type (Sappino et al., 1990; Schürch et al., 1992).

Myofibroblasts are the main cellular type involved in extracellular matrix deposition during wound healing (Zhang et al., 1994). To replace the damaged tissue, myofibroblasts participate actively to the synthesis of extracellular matrix components such as tenascin, fibronectin, and collagens I and III (Desmoulière et al., 1997b). It is important to emphasize that a subtle balance exists between matrix synthesis and matrix degradation during wound healing, and usually the same cells responsible for extracellular matrix deposition are also responsible for the synthesis of enzymes involved in matrix degradation such as metalloproteinases (MMPs), including collagenases, gelatinases and stromelysins (Murphy and Docherty, 1992), and by the synthsesis of tissue inhibitors of metalloproteinases (TIMPs) (Norman and Lewis, 1996). These processes are strongly regulated by growth factors and cytokines.

Factors involved in myofibroblastic differentiation

Cytokines

The application of granulocyte macrophage-colony stimulating factor (GM-CSF) to the rat subcutaneous tissue induces the formation of an important granulation tissue rich in α-SM actin positive myofibroblasts (Rubbia-Brandt et al., 1991). In vitro experiments have shown that GM-CSF does not directly stimulate α-SM actin expression when added to the culture medium of rat or human fibroblasts. Indeed, after GM-CSF local treatment, the appearance of α-SM actin-rich myofibro-blasts (Rubbia-Brandt et al., 1991) is preceded by a characteristic cluster-like accumulation of macrophages (Vyalov et al., 1993), suggesting that such macrophages are important in the stimulation of α-SM actin synthesis by myofibroblasts. During the early steps of pulmonary fibrosis development after intra-alveolar installation of bleomycin, GM-CSF is expressed by inflammatory cells (Andreutti et al., 1998). Moreover, GM-CSF induces the expression of transforming growth factor-ß1 (TGFß1) mRNA by alveolar macrophages (Andreutti et al., 1998). These data support the possibility that GM-CSF participates in the initial steps of the chain of events leading to fibrosis, perhaps through a stimulation of TGFß1 production. Indeed, among factors secreted by activated macrophages and capable of modulating the expression of α-SM actin, TGFß1 is probably the most efficient (Desmoulière et al., 1993). We have shown that TGFß1 stimulates the expression of α-SM actin in granulation tissue myofibroblasts (Desmoulière et al., 1993). Furthermore, the expression of α-SM actin protein and mRNA by TGFß1 is induced in both growing and quiescent cultured fibroblastic populations. The action of TGFß1 on α-SM actin expression confirms and extends the notion that TGFß1 plays an

important role in both fibroblast differentiation and fibrosis formation. Recently, Serini and Gabbiani (1996) have shown that (1) TGFß2, like TGFß1, induces myofibroblast formation in vivo and in vitro; (2) TGFß3 acts as a negative regulator of the myofibroblastic phenotype in vivo but not in vitro; and (3) in vitro, the three different TGFß isoforms are equally able to induce α-SM actin mRNA and protein expression in growing and quiescent cultured human and rat subcutaneous fibroblasts. These data confirm that in vitro the behaviour of the three different TGFß isoforms is similar, whereas in vivo TGFß isoforms possible play different but complementary roles in myofibroblast modulation during wound repair.

In cultured fibroblasts, γ-interferon (γIFN), a cytokine produced by T-helper lymphocytes, decreases α-SM actin protein and mRNA expression as well as cell proliferation (Desmoulière et al., 1992a). Preliminary results (Pittet et al., 1994) have shown that γIFN treatment decreases the symptoms and the size of hypertrophic scars and Dupuytren's nodules. In hypertrophic scars, immunofluorescence examination showed that α-SM actin expression was decreased in myofibroblasts of treated lesions. Furthermore, it has been shown that αIFN decreases α-SM actin expression of human hepatic stellate cells in culture (Mallat et al., 1995) and reduces liver fibrosis in non-A, non-B hepatitis (Manabe et al., 1993). These results suggest that IFNs could represent a useful adjunct to the nonsurgical therapy of some fibrocontractive diseases. Recently, we have shown that in two animal models of liver fibrosis induced by either carbon tetrachloride or bile duct ligation, pentoxifylline, a methyl xanthine phosphodiesterase inhibitor, is able to reduce fibro-proliferation and myofibroblastic differentiation and appears as a potential antifibrogenic drug (Desmoulière et al., 1999).

Platelet-derived growth (PDGF), basic fibroblast growth factor and tumor necrosis factor-α (TNFα) appear to decrease the expression of α-SM actin mRNA and protein in cultured fibroblasts (A. Desmoulière and G. Gabbiani, unpublished observations) but do not induce myofibroblastic differentiation in vivo (Rubia-Brandt et al., 1991).

Extracellular matrix components

It is well accepted that the extracellular matrix represents a structural support for cellular constituents but evidence exists showing that the matrix plays a central role as a source of signals influencing growth and differentiation of different cell types, including fibroblasts (for review, see Juliano and Haskill, 1993). Among extracellular matrix components, different types of collagen, glycoproteins and proteoglycans are involved in fibroblastic differentiation. In fibroblasts cultured in the presence of fetal calf serum and in vivo, when cells proliferate, heparin increases the expression of both α-SM actin protein and mRNA (Desmoulieère et

al., 1992b). Fibronectin is a prominent matrix component that promotes cell migration in development and has important functions in several stages of wound healing. Molecular cloning has demonstrated that fibronectin exists in multiple forms that arise from a single mRNA transcript that can be alternatively spliced in three regions: EIIIA (ED-A), EIIIB, and V. During experimental hepatic fibrosis induced by ligation of the biliary duct, it has been shown that the fibronectin isoform ED-A secreted by sinusoidal endothelial cells mediates the conversion of hepatic stellate cells to myofibroblasts (Jarnagin et al., 1994). During cutaneous wound healing, both macrophages and fibroblasts express ED-A- and EIIIB-fibronectin mRNA (Brown et al., 1993). Recently, Serini et al. (1998) have shown that the fibronectin domain ED-A is necessary for the induction of the myofibroblastic phenotype by TGFß1. Furthermore, we have observed that, in the experimental model of liver fibrosis induced by common bile duct ligation, fibroblasts within the portal tracts proliferate and acquire α-SM actin. Moreover, extracellular matrix deposition occured very early and preceded myofibro-blastic differentiation, suggesting that myofibroblastic differentiation represents an adaptative response to modifications of the extracellular matrix environment (Desmoulière et al., 1997b).

Remodelling of granulation tissue

During the repair of injured tissues, the mass of granulation tissue must be controlled and limited to prevent an anarchic remodelling and the development of fibrosis. The final process in normal wound healing is resolution of the scar. For example, it has been shown that during the healing of an open wound, there is an augmentation in the amount of cells expressing α-SM actin which disappear after the wound is closed (Darby et al., 1990). The reduction of the granulation tissue involves an apoptotic process. In late phases of wound healing, many myofibroblasts show changes compatible with apoptosis observed by electron microscopy and by in situ labeling of fragmented DNA (Desmoulière et al., 1995). Thus, apoptosis is one of the mechanisms for the resolution of granulation tissue after tissue injury. When a disturbance occurs, the myofibroblasts will not disappear and will continually deposit extracellular matrix, which leads to development of fibrosis.

Different renal fibroblastic populations

For a long time, the fibroblastic population was considered as a homogeneous cell population of connective tissue cells widely distributed in the different organs. But in the last twenty years, many cells called "fibroblast-like cells" have been described, and an in-depth study in these fibroblast-like cells pointed out that they were, in fact, different subpopulations of fibroblast with specialized functions (Komuro, 1990). In the kidney, at least seven (extraglomerular and glomerular

mesangial cells, cortical and medullary interstitial cells, dendritic cells, pericytes, lipid-laden interstitial cells) different subpopulations of fibroblastic cells are present. It is important to consider these different subpopulations when pathological processes are described. This point has been particularly highlighted in the liver, where two different fibroblastic cell populations can be involved during fibrogenesis; following chemically-induced injury, the hepatic stellate cell is the main component of the fibrogenic cell population, while in cholestatic injury, portal periductular fibroblasts are primarily involved in early portal fibrosis. The mechanisms of activation of these fibroblastic subpopulations are different and must be considered when evaluating the hepatoprotective and antifibrogenic potential of drugs (Desmoulière et al., 1999).

Mesangial cells

The glomerular mesangial cells are, in fact, a very particular type of fibroblast, that may be located inside or outside the glomerulus. Extraglomerular mesangial cells (Polkissen cells or polar cushion) form a cushion of cells between the walls of afferent and efferent arterioles and are a part of the juxtaglomerular apparatus (Ross and Romrell, 1989). Extraglomerular mesangial cells contain granules of renin and many filaments in the cytoplasm, beyond the usual organelles. Intraglomerular mesangial cells are contiguous to the extraglomerular ones. Intraglomerular mesangial cells are located betwen the loops of the glomerular capillary and resemble pericytes. These cells have a small, dense nucleus, fine filaments specially abundant along the cell membrane, and long processes, some of which penetrate the mesangial matrix underlying the capillary endothelium. Mesangial cells have many functions: phagocytosis of proteins which are retained on basement membrane during filtration; structural support for the glomerulus on basement membrane during filtration (Lovett and Sterzel, 1986); proinflammatory effector cells capable of release cytokines such as tumor TNF (Baud et al., 1989) and interleukin-6 (Horii et al., 1989). Mesangial cells are considered to have a common origin with vascular SM cells, since they are contractile, a function that may probably modulate glomerular hemodynamics by controlling glomerular capillary surface area (Schlondorff, 1987). However, mesangial cells do not express α-SM actin in normal conditions. In culture, they acquire the expression of in α-SM actin (Elger et al., 1993). When the cells are grown on the usual plastic they develop adherences and extensions, and typical stress fibers can be observed which allow contraction (Schlondorff, 1987). Contraction and relaxation of cultured mesangial cells have been observed in response to a number of agents (Singhal et al., 1986). Mesangial cells are also involved in the synthesis of extracellular matrix in response to mechanical stress, as has been shown on cultured mesangial cells subjected to repetitive cycles of stretch/relaxation (Riser et al.,1992).

Interstitial cells

Renal interstitium may be divided into cortical and medullary compartments. Renal interstitial cells consist of a heterogeneous cell population and include fibroblast-like cells, lipid-laden interstitial cells, pericytes and dendritic cells (Lemley and Kriz, 1991). The fibroblasts represent the most frequent interstitial cell type in the cortex and in the inner medulla, the unique interstitial cell type (Kaissling and Le Hir, 1994). Fibroblasts of the cortex and of the inner medulla are similar in their ultrastructure. They are characterized by long, branching processes which come close to vessels and tubules and, in some circumstances, appear to encircle them. Medullary interstitial fibroblasts were classified by Komuro (1990) as secretory interstitial cells, as their most distinctive feature is the presence of numerous lipid droplets which contain a pro-hormone with anti-hypertensive activity. Beyond the endocrine function, these cells have two other functions: mechanical support and production of ground substances in the surrounding interstitium. Furthermore, cortical and inner medullary fibroblasts possess the ability to transform into myofibroblasts (Grupp et al., 1997).

Fibroblasts and dendritic cells represent the bulk of interstitial cells in the cortex. The morphological characteristics of these cells appear quite similar at first glance and cannot be easily discriminated. However, electron microscopy can show distinctive ultrastructural features. Cortical fibroblasts possess numerous large cytoplasmic extensions and well developed rough endoplasmic reticulum. The perikaryon is often completely occupied by the nucleus. Specialized junctions can be observed between these cells as well as with the basement membranes of capillaries and of tubules, which suggests the importance of these cells in regulative and pathological mechanisms (Kaissling and Le Hir, 1994). Dendritic cells express the MHC II antigens and are probably identical to the dendritic cells described in various tissues (Steinman, 1991). In contrast to cortical fibroblasts, the nucleus is surrounded by a rather large rim of cytoplasm containing mitochondria and characteristic profile of endoplasmic reticulum which is often irregularly lined by ribosomes. Furthermore, no junctions between dendritic cells or with either tubules or capillaries have been observed. The relatively scarce rough endoplasmic reticulum does not suggest any role of these cells in the production of extracellular matrix.

Pericytes are particularly abundant in the medulla, where they surround the descending vasa recta. Pericytes are enclosed by a basement membrane and are often considered to be a transitional cell type between vascular SM cells and fibroblasts. α-SM actin expression was found specifically in pericytes surrounding descending vasa recta within the outer and inner medulla (Park et al., 1997).

Lipid-laden interstitial cells contain numerous osmiophilic lipid droplets and have an abundant rough

endoplasmic reticulum and a well developed cyto-skeleton. They have specialized composite junctional connections to each other but not to the capillaries, from which they are separated by a basement membrane (Lemley and Kriz, 1991). In cell culture, it has been shown that these cells possess receptors for angiotensin II (Brown et al., 1980), suggesting a possible role in contraction.

Glomerular events in renal diseases

Many glomerular diseases are the result of immune injury mediated by macrophages and lymphocytes where antibodies directed against glomerular antigens and deposition of antigen-antibody complexes in glomerular basement membranes initiate the inflammatory process mediated by various cytokines and growth factors. Some glomerular diseases like those caused by hypertension involve hemodynamic stimuli mediated by humoral mediators such as angiotensin II. The common result of these different glomerular diseases is glomerulosclerosis which is characterized by the excessive accumulation of extracellular matrix by activated fibroblasts (myofibro-blasts) in response to various inflammatory or hemo-dynamic stimuli. Expression of α-SM actin in glomeruli is indicative of mesangial cell activation resulting in the accumulation of extracellular matrix components.

Experimental models

In the anti-Thy 1 model of mesangial proliferative glomerulonephritis, the injection of antibody to the Thy1 antigen induces an early phase of mesangiolysis accompanied by a glomerular platelet infiltrate. These events are followed by a phase of mesangial cell proli-feration and the differentiation of these cells into myo-fibroblasts with an abundant deposition of extracellular matrix components (Johnson et al., 1991). This model resembles the morphological features of human glomerulonephritis. In this model, lesions are transient and cell number returns spontaneously to normal within 3 weeks (Baker et al., 1994). It has been shown that the resolution of glomerular hypercellularity is mediated by mesangial cell apoptosis (Baker et al., 1994; Shimizu et al., 1995).

The most studied model of progressive glomerulo-sclerosis leading to end-stage renal disease is the sub-total nephrectomy in rats. In this model, an early proliferative wave of mesangial cells takes place followed by macrophage influx and differentiation of mesangial cells into myofibroblastic cells (Muchaneta-Kubara and El Nahas, 1997).

Human diseases

Injury to the mesangium is common in many glomerular diseases including some characterized by immune complex depositions (IgA nephropathy, membranoproliferative glomerulonephritis, lupus nephritis) and some diseases without a prominent inflammatory cell involvement (diabetic nephropathy, nephroangiosclerosis, some forms of focal and segmental glomerulosclerosis). There is a considerable heterogeneity in the natural history of the disease process in each of the categories but the result is the activation of mesangial cells which acquire the expression of α-SM actin. Since mesangial cells express α-SM actin only in pathological situations, the enhanced expression of α-SM actin is a sensitive marker of mesangial injury (activation) that may be detected in fixed tissue sections.

A marked increase of α-SM actin expression is common in many forms of glomerular injury, such as proliferative glomerulonephritis (lupus and focal glomerulosclerosis) (Alpers et al., 1992) or non proliferative glomerulonephritis (membranous nephro-pathy and diabetic nephropathy) (Muchaneta-Kubara and El Nahas, 1997; Roberts et al., 1997). Acute glomerulo-nephritis with cellular proliferation and influx of inflammatory cells or slowly progressive processes such as diabetic nephropathy or membranous nephropathy can result in equivalent levels of α-SM actin. Glomerular scar is known to contain not only type IV collagen but interstitial type III collagen as well. It has been suggested by Striker et al. (1984) that the presence of type III collagen in glomeruli results from the migration of interstitial cells into the glomeruli through breaks in basement membrane of Bowman's capsule. However, in primary focal segmental glomerulosclerosis, it has been shown that de novo synthesis of type III collagen is present in glomeruli with intact Bowman's capsules and that type III collagen accumulation in glomeruli results from the activation of mesangial cells into myofibro-blasts expressing α-SM actin (Hattori et al., 1997). Little is known about the role of the myofibroblast contractile capacities in renal diseases. However, there is evidence that cells which express contractile elements such as α-SM actin, probably play an important role in renal hemodynamic (MacPherson et al., 1993).

Interstitial events in renal diseases

The development of tubulointerstitial fibrosis correlates closely with the loss of renal function in patients with chronic glomerular disease (Bohle et al., 1996). However, mechanisms involved in the development of tubulointerstitial fibrosis are not well understood. Many morphometric studies indicate that tubulointerstitial changes correlate better with reduced renal function than glomerular changes (Bohle et al., 1996; Ziyadeh, 1996). Interstitial myofibroblasts play an important role in the pathogenesis of tubulointerstitial fibrosis.

Experimental models

Tang et al. (1997), combining immunohisto-

chemistry and in situ hybridization, showed, in a model of experimental tubulointerstitial nephritis associated with muromycin aminonucleoside nephrosis, that the myofibroblasts were the principal cell type responsible for the increased expression of $\alpha1(III)$ collagen mRNA in the early phase of injury. Furthermore, the administration of PDGF-BB to rats induces the differentiation of tubulo-interstitial myofibroblasts and the expression of $\alpha1(III)$ collagen mRNA in these cells (Tang et al., 1996), suggesting that PDGF-BB may be an important mediator of tubulo-interstitial hyperplasia and fibrosis.

In the model of subtotal nephrectomy, cells expressing α-SM actin were detected as early as the first week in the interstitium preceding the development of tubulointerstitial fibrosis. They increased with time, surrounding the glomeruli and around the tubules (Muchaneta-Kubara and El Nahas, 1997).

Human diseases

In normal human kidney tissue, α-SM actin-positive cells were essentially confined to the SM cells of arteries and arterioles. In the kidneys of patients with diverse glomerular diseases, the distribution of interstitial myofibroblasts varied, depending on the severity of the nephropathy (Fig. 1). In the interstitium, the myofibroblasts were localized in the peritubular and periglomerular spaces.

The origin of these interstitial cells is still a matter of controversy. Some have implied that they are derived from vascular SM cells and pericytes and others have suggested that they are activated resident interstitial fibroblasts (Gabbiani, 1992; Wiggins et al., 1993; Boukhalfa et al., 1996). Recent findings support an alternative hypothesis that fibroblasts may arise from epithelium through a phenomena called epithelial-mesenchymal transdifferentiation (Okada et al., 1996). This process has been described in cell culture systems, where epithelial cells lose polarity, adhere to adjacent cells and basal lamina, convert into fusiform shapes, and gain mesenchymal properties including motility. Recently, it has been suggested in vivo for the model of subtotal nephrectomy, that tubular epithelial cells lose apical-basal polarity and tight junctions, become elongated, detach from the tubular basement membrane, separate from neighboring cells and appear to migrate into the peritubular interstitium through the damaged basement membrane (Ng et al., 1998).

Interest of myofibroblast evaluation in renal biopsies

Standard histological parameters usually cannot discriminate at the onset between patients with stable or declining renal function. For example, the clinical course of membranous nephropathy is unpredictable and it is difficult to assess the prognosis when the diagnosis is done. Stages of disease, classified by Ehrenreich and Churg (1968), seem to have no influence in the

prognosis of membranous nephropathy. Some patients will have a clinical remission and others will progress slowly to renal failure, making the selection of patients for an early treatment which may sometimes present undesirable side effects difficult (Cameron, 1992; Couchoud et al., 1994). Many clinical predictors of disease progression, such as the level of proteinuria or renal impairment at presentation, have been reported (Braden et al., 1988; Cattran et al., 1997). The validation of early predictor factors and particularly histological parameters would allow a better definition for the treatment of patients at risk. Scarring within the interstitium seems to be important in the long term renal outcome (Bohle et al., 1996) and the interstitial fibrosis on renal biopsy is predictive of renal function impairment in many glomerulonephritis (Nath, 1992). However, fibrosis is a late event which often corresponds to an irreversible scar and is often associated with renal function impairment on diagnosis. We have studied the relationship between α-SM actin expression and renal outcome in biopsies of patients with membranous nephropathy, and we have found a strong correlation between the amount of interstitial myofibroblasts in biopsies at diagnosis and the outcome of the disease (Badid et al., 1999). Recent studies have also shown a correlation between the amount of interstitial myofibroblasts and the renal function at the time of biopsy in IgA nephropathy (Goumenos et al., 1994) and in diabetic nephropathy (Essawy et al., 1997).

These observations support the interest in kidney diseases of the myofibroblast differentiation study which can help to discriminate between progressors and non-progressors. We suggest that myofibroblast evaluation on renal biopsies could complete the clinical prognostic values of conventional parameters. For evidence, early cellular events in the development of chronic renal failure can be observed histologically in many glomerular diseases before renal function declines and fibrosis develops. The differentiation into myofibroblasts involved in the synthesis of extracellular matrix components can be considered as an early event before the development of fibrosis and can represent an important marker in the assessment of many glomerulo-pathies. It could be interesting to validate a reliable semiquantitative method by using myofibroblastic markers, to identify patients at risk for renal function declining in membranous nephropathy and in other nephropathies as well since myofibroblastic modulation seems to be a pivotal event during the pathological evolution of inflammation which contributes to subsequent fibrosis.

Perspectives: myofibroblast as a target cell

Scarring of tissues and organ fibrosis represent a medical challenge. Growing understanding of the cellular and molecular events involved in fibrosis development provides an interesting basis for development of specific drug therapies. Much attention
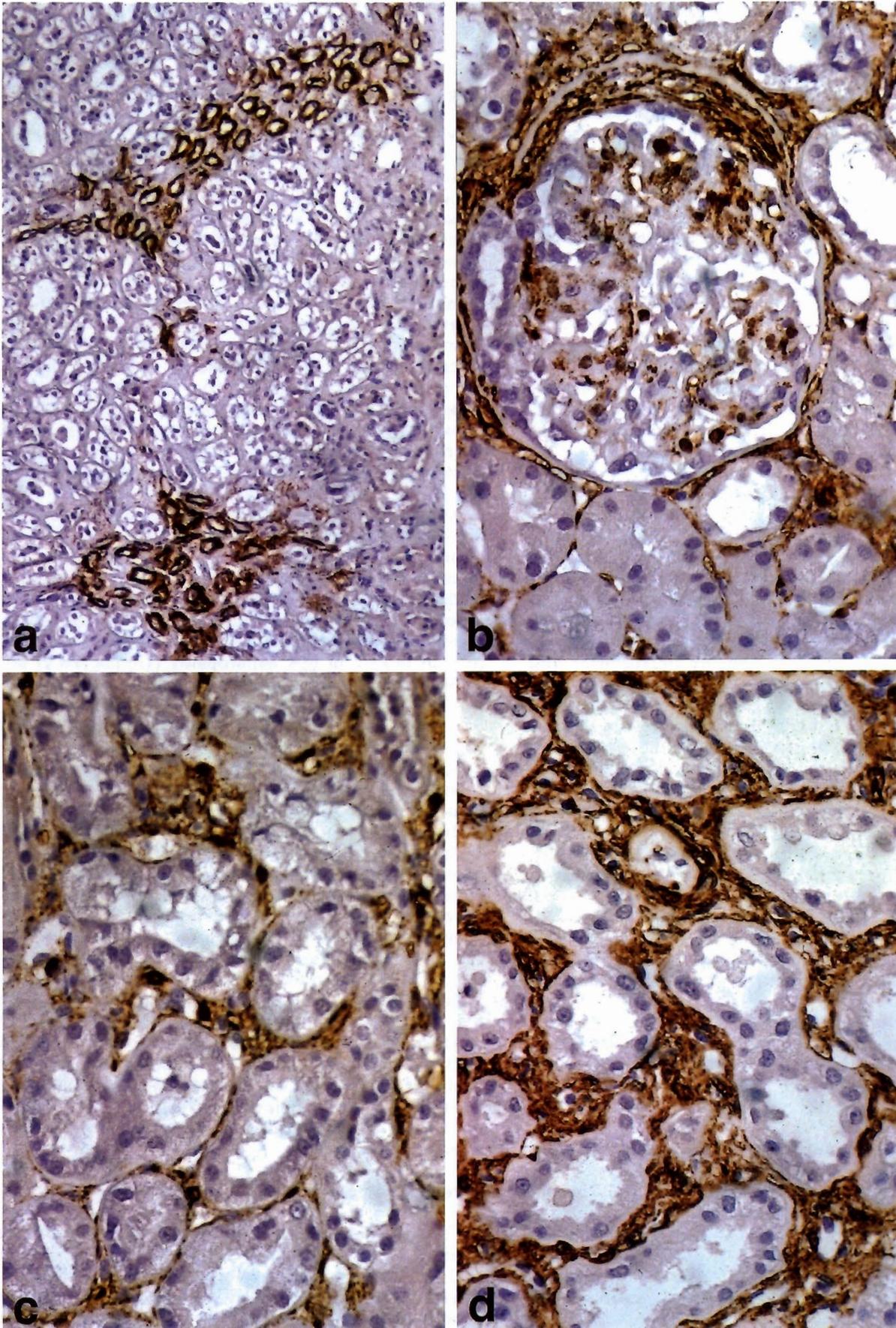
Fig. 1. α-SM actin expression in different situations. **a.** α-SM actin expression within renal medulla, surrounding vasa recta. **b and c.** Glomerular and interstitial α-SM actin expression in a 45-year old-man with membranous nephropathy who developed renal insufficiency in 4 years; renal function was normal at diagnosis. A thin layer of α-SM actin-positive cells can be seen around most of the tubules and around the glomerulus. Mesangial cells express α-SM actin as well. **d.** Interstitial α-SM actin expression in a 49-year-old man with membranous nephropathy who rapidly developed renal insufficiency in 1 year; renal function was normal at diagnosis. All the tubules are surrounded by several layers of α-SM actin-positive cells. a, x 400; b-d, x 800

was focused on the roles of some cytokines (TGFß1, PDGF), which contribute to the fibrogenic process, and to the interest of blocking the activation of latent TGFß1 or neutralizing the PDGF. In the Thy 1 glomerulo-nephritis model, blocking TGFß1 by infusing neutralizing antibodies (Border et al., 1992) or blocking local synthesis by the intrarenal perfusion of antisense oligonucleotides (Akagi et al., 1996) suppresses extracellular matrix accumulation. The fibrogenic effects of endothelin and angiotensin II have aroused considerable interest in the anti-fibrotic potential of anti-hypertensive agents such as angiotensin-converting enzyme inhibitors. In fact, angiotensin II is known to stimulate collagen deposits by mesangial cells associated with the synthesis and activation of TGFß (Wolf et al., 1992).

New ways of treatment should focus on the modulation of myofibroblastic cell activation and proliferation, and their eventual death by apoptosis (Shimizu et al., 1995; Desmoulière et al., 1997a) can represent a potentially limiting clearance mechanism.

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